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# A REMARKABLE POLYMORPHISM OF MATURE LARVAE OF *ZIZINA LABRADUS* (GODART), COMMON GRASS BLUE BUTTERFLY (LEPIDOPTERA: LYCAENIDAE) FROM THE SYDNEY AREA

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## Abstract

Polymorphism in body colour of mature larvae in *Zizina labradus* (Godart) ranging from white to red, green and dark purple became apparent upon rearing larva on an artificial diet. In field populations this polymorphism seems to be largely obscured by the dominant green colour which seems to be derived from the colour of the foodplant. Initial attempts to select for the white, red and green colour of the larvae were unsuccessful. The frequency of the red larvae increased significantly at the expense of the white and other pale-coloured larvae when the larvae were reared under red or green ambient light, whereas yellow and blue ambient lights had no effect. I suggest that the phenotypic decision of red *versus* white in the larval body colour may be made epigenetically, although some genetic predisposition to any body colour is not ruled out.

## Introduction

Currently I am using *Zizina labradus* (Godart) [alternatively, *Zizina otis labradus* (Godart)] as experimental material in developmental biology, and rear the larvae on an artificial diet. By so doing I have uncovered extensive polymorphism in the body colour of the mature larvae of this species, which seems to be largely obscured in field populations owing to the intense green colour of the larvae, presumably resulting from chlorophyll in the foodplant. The breeding of this species on the artificial diet is easy. A systematic approach

to the phenomenon of colour polymorphism thus revealed may yield valuable information about the nature of larval polymorphism in lycaenid butterflies.

Observations reported in this paper are a research by-product and only preliminary. I do not at the moment intend to pursue the subject further.

## Materials and Methods

*Butterflies.* Local populations in North Ryde, Lindfield, Wahroonga and Seaforth (all Sydney suburbs) were used, with a population at Mount Wilson, Blue Mountains, New South Wales, as additional material.

*Artificial diet.* This is essentially based on the recipe of Shorey and Hale (1965): mix Solution A containing 25 g lentil flower (*besan* used in Indian and Burmese cooking), 7 g dry yeast, 3.1 g alfalfa meal (optional), 0.7 g ascorbic acid (stored in a deepfreezer), 0.44 g Nipagin M (a fungicide) and 0.22 g sorbic acid in 70 ml water with Solution B containing 2.8 g agar-agar dissolved in 70 ml boiling water, cool to 60°C and then stand in a 60° water bath to prevent solidification. Portions of this mixture are poured into obliquely held, transparent, plastic breeding-jars (6.7 cm inner diameter, 7.8 cm deep) with white or blue, non-transparent screw-lids, wetting one side of the wall and about two-thirds of the bottom; excess liquid may be poured back to the mother mixture. The jars are cooled and dried by standing at room temperature for one hour and the lids replaced. The jars thus prepared can be stored at room temperature for several weeks. Storage in a refrigerator or freezer is not practical because of the heavy condensation. The young larvae usually settle on the dry plastic surface at the edge of the food-layer on the upright side wall and feed. Alternatively, the solutions may be mixed and poured into a tray, loosely covered and left overnight, then shredded, further dried again overnight, and finally stored in jars in a refrigerator (see Morton, 1979). In this way the diet can be kept for up to several months and used in small portions as required.

*Breeding.* Female butterflies, freshly collected in the field in early afternoon, were brought back to the laboratory and up to 10 insects were put in a transparent, plastic cylinder with metal ends (20 cm diameter and 31.5 cm high; Australian Entomological Supplies) under illumination with eleven 20W fluorescent lamps held vertically in a U-shaped array for a period of 12 hours (0500-1700). Because of the lamps the temperature of the oviposition chamber rose to 29°C in a room at 25-27°; overheating was avoided by appropriately adjusting the distance of the chamber from the lamps. Sucrose solution (5%) in a small beaker holding a fluted filter paper and 3-6 stalked clover leaves (foodplant) in 1-2 watered flask/s were provided. Oviposition (mostly on the underside of the leaves) was very active on the day following capture, yielding up to 35 eggs per female under favourable conditions, but both egg laying and egg viability declined on later days.

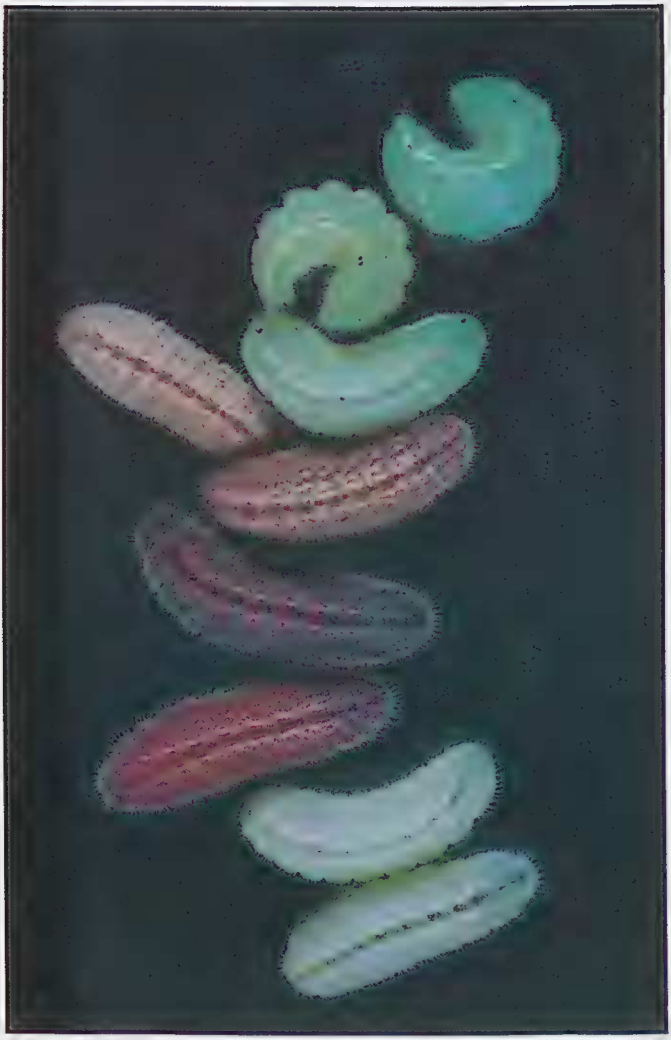

Fig. 1. Selected examples of various body colours in mature larvae of *Zizina labradus* (Godart) from Sydney area (x 4.1).

The eggs were dislodged with a small spatula and collected in small plastic Petri dishes (60 mm). If necessary, they were counted under a dissecting binocular microscope on a section paper fitted to the lid of a Petri dish. Immediately prior to hatching, a fresh clover leaf was placed in the Petri dish, or the dish containing eggs was placed in the lid of the breeding jar standing upside down to let newly hatched larvae crawl up to the food. The

procedures seemed to affect only slightly the viability of the egg, which was 86-98% under optimum conditions.

The newly hatched larvae were placed in rearing jars with a very small paint brush at a desired density (20 per jar was adequate) and held at 23°. Cannibalism was reduced at this temperature by keeping the lid loose to lower humidity (Morton, 1979) from the third instar on, and also by reducing the population density to 10, 5 or even less per jar with the growth of the larvae. The young larvae were thus gradually thinned out by transferring to new jars after 1-2 weeks, especially when they moulted on the lid. Alternatively, fresh pieces of shredded diet were attached to the side wall of the jar whenever food became dry or scarce. The egg, larval and pupal stages lasted 5-6, 32 and 8-10 days, respectively, and about 50% of the larvae survived to produce adults. Viability could be increased to above 80% by rearing each larva separately.

*Mating.* Artificial lights were largely insufficient to elicit mating behaviour with this species. When placed in a wire cage measuring $(60\,cm)^3$, the butterflies mated readily (mostly within 30 minutes) upon exposure to sunlight (usually in the late morning) in the absence of strong draught. Copulation lasted for about 30 minutes. Females started laying eggs on the second day after copulation. I suspect that females prevented from vigorous flying tended to yield less viable eggs.

## Results

The young larva was colourless, but started to show the first sign of colour patterns, consisting of the median dorsal and other longitudinal lines, at the second instar. Thereafter the larvae gradually acquired body colour and enhanced patterning. These patterns aside, the body colour of larvae diverged gradually and, at the fifth instar, ranged from an almost pure, even sometimes shiny, white, through light grey-brown, pale red, wine red to dark purple, and also through pale green and green to almost shining emerald or bluish green. Examples are shown in Fig. 1. These colours appear to be largely due to pigmentation of the epidermis, but the emerald green larvae (and pupae; see below) apparently had green haemolymph. The dark-purple colour may be due to a combination of the red epidermis and green haemolymph, but this point was not ascertained. The cuticle did not contribute to the colour. The Blue Mountain population showed a slightly different trend of patterning with many individuals having more pronounced pale areas on both sides of the dorsal median line compared with those from the Sydney population.

The proportion of various colour-forms was roughly stable in the Sydney population, intermediate ones usually predominating. The green and

red colours were carried over in early pupal stages. Pupae also varied in the intensity of dark stripes and brown dusty stipples. I have so far not found any correlation between adult sex or phenotype and larval colour type.

Attempts were made to select for white, red, dark purple and green larvae, but the $F_1$ and $F_2$ failed to show any effect of selection. Rather the parental phenotypes tended to be reduced in proportion among the progeny.

The possible effect of environmental factors on body colour was therefore tested. First, newly hatched larvae of mixed family lines were placed in coloured plastic containers with translucent lids. A green container yielded all green larvae and a red one yielded all red larvae, while yellow and beige containers gave mixed populations. But the small sample size precluded any definite conclusion. In another experiment, eggs and rearing jars containing larvae were placed inside plastic rearing cylinders with transparent plastic side walls covered with coloured cellophane sheets. Red, yellow, green and blue cellophanes were tested together with a control without cover under illumination of two 40W horizontal fluorescent lamps from 0500 to 1700 (Eastern Standard Time) but with lingering summertime daylight and room lights for night cleaning for several hours more. The results shown in Table 1 apparently indicate that the frequency of red body colour increases at the expense of white and other pale body colours under the influence of red and green ambient lights, but the yellow and blue ambient lights do not share this effect. The green body colour, on the other hand, does not seem to be enhanced by any colour of the ambient light. Of course, these results are only very preliminary and need verification with more carefully and systematically designed experimentation. But generally speaking, the appearance of the body colour seems to be environmentally affected and therefore epigenetically regulated, rather than genetically controlled as a first approximation, at least in part.

TABLE 1

Frequency of each different body colour in mature larvae of *Zizina labradus* reared on artificial diet under ambient lights of different colours (frequency shown as a percentage of the total number of larvae).

| Colour of larva | Colour of ambient light | | | | |
|---|---|---|---|---|---|
| | Control (White) | Red | Yellow | Green | Blue |
| White | 15% | 0% | 13% | 0% | 21% |
| Grey-brown | 15 | 0 | 13 | 3 | 26 |
| Pale green | 8 | 7 | 13 | 7 | 5 |
| Green | 15 | 20 | 25 | 7 | 16 |
| Pale red | 15 | 0 | 21 | 0 | 5 |
| Red | 23 | 60 | 17 | 83 | 26 |
| Dark purple | 8 | 13 | 0 | 0 | 0 |
| Total number of larvae | 13 | 15 | 24 | 30 | 19 |

For experimental conditions, see text. Test for overall heterogeneity of the sample: $\chi^2 = 54.46$ ($P < 0.01$).

## Discussion

Extensive larval polymorphism in a natural population of an Australian lycaenid [*Theclinesthes albocincta* (Waterhouse)] has been reported by Grund and Sibatani (1975). Some polymorphism in natural populations of *Z. labradus* larvae in Australia has also been noted by Common and Waterhouse (1972, 1981) and Fisher (1978), who state that the larvae are of "various shades of green".

In populations of *Z. labradus* larvae feeding on clover, the colour variation appears to be rather limited because they are all green in body colour, with occasional variants of dark coloration presumably corresponding to the wine red or dark purple form found among larvae raised on the artificial diet. A series of research projects, requiring little instrumentation, could be undertaken on the genetic, epigenetic, ecological and biogeographical aspects of the polymorphism. Moreover, such studies could be combined with isozyme analyses now widely used in population genetics. An important point would be to ascertain whether or not the colour polymorphism of the larva as reported here is a "natural" phenomenon that is largely obscured by the chlorophyll of the food plants or a phenomenon actually induced by the artificial diet employed here. A further interesting question would be to determine the selective significance of the polymorphism which is largely obscured or, in the case of green forms, apparently made redundant by the green colour contained in the natural food plant.

The same artificial diet as used here was accepted by all newly hatched larvae of *Candalides absimilis* (Felder) (Lycaenidae) from eggs laid on wisteria in the Sydney area. Only a minority of them survived to emerge as normal adults and so far no larval polymorphism has been detected.

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